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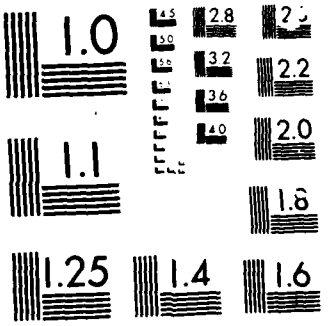

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AD-A165 725

STEREOELECTRONIC CONTROL IN THE PHOSPHORYLATION OF SERINE ESTERASES

FINAL REPORT

DAVID G. GORENSTEIN

2/6/86

U. S. ARMY RESEARCH OFFICE

CONTRACT/DAAG29-83-K-0098

PURDUE UNIVERSITY
WEST LAFAYETTE, IN 47907

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Final Report	2. GOVT ACCESSION NO. AD-A165725	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Stereoelectronic Control in the Phosphorylation of Serine Esterases		5. TYPE OF REPORT & PERIOD COVERED Final 8/1/83-7/31/85
		6. PERFORMING ORG. REPORT NUMBER 376000511
7. AUTHOR(s) David G. Gorenstein		8. CONTRACT OR GRANT NUMBER(s) DAAG29-83-K-0098
9. PERFORMING ORGANIZATION NAME AND ADDRESS Purdue University West Lafayette, IN 47907		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Research Office Post Office Box 12211 Research Triangle Park, NC 27709		12. REPORT DATE 02/06/86 13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

NA

18. SUPPLEMENTARY NOTES

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Organophosphorus, Reactivity, Stereoelectronic Effect, Nucleophilicity, Hydrolysis, Molecular Orbital Calculations

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Our research has involved several approaches to the study of stereo-electronic control in the enzymatic reactions of organophosphorus compounds. This stereoelectronic control involves weakening of a P-O ester bond by antiperiplanar interactions with oxygen or nitrogen lone pairs. Our first approach has been to study the kinetics and mechanism of enzymatic reaction of a number of conformationally restricted phosphorus compounds to determine the importance of stereoelectronic effects in enzymatic phosphorylation at phosphorus. The

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stereochemistry of reaction of phosphate esters has extended to the non-enzymatic and enzymatic reactions of phosphate triesters. ^{31}P NMR has been used to monitor the stereochemistry of the enzymatic covalent modification as well as the reactivation and aging reactions of covalently phosphorylated enzymes. Ab initio molecular orbital calculations have also revealed important stereoelectronic effects.

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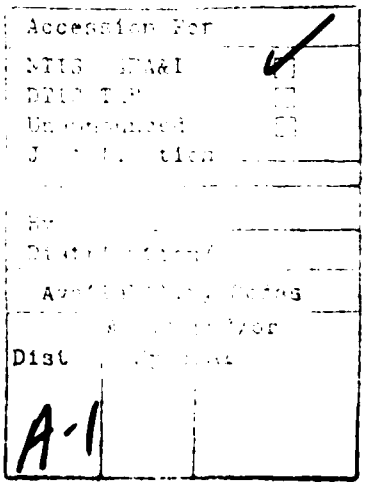


5. Final Report

a. Problem Studied

This research presented several rather different approaches to the study of stereoelectronic and stereochemical effects in the enzymatic reactions of organophosphorus compounds. The major emphasis in our study involved work with neutral phosphate simulants that have little neurotoxicity. However, the basic understanding that we hoped to develop on stereoelectronic effects have important ramifications for the mechanism of neurotoxicity and for the mechanism of detoxification of chemical agents, since recently we have shown that stereoelectronic effects can potentially greatly alter the reactivity of phosphate esters. This stereoelectronic effect involves activation of a P-O ester bond by antiperiplanar interactions with oxygen or nitrogen lone pairs. We studied the kinetics and mechanism of nonenzymatic and enzymatic reaction of a number of conformationally restricted phosphate triesters and related compounds. These studies help demonstrate the importance of stereoelectronic control in the enzymatic reactions of cyclic and acyclic phosphorus compounds.

Stereochemical studies have traditionally been one of the most important mechanistic probes available to the physical organic chemistry. Only recently have chiral phosphate esters (using oxygen isotopes) become available and with a new ^{18}O-^{31}P shift probe of configuration that we helped pioneer, rapid progress in describing the stereochemistry of enzymatic reactions of phosphate esters was now feasible. Understanding the stereochemistry of the covalent phosphorylation of serine esterases such as α-chymotrypsin allowed us to better map the active site geometry of these enzymes. We are now also in a position to determine the stereochemistry of the reactivation and aging reactions of these organophosphate inhibited enzymes. All of these stereochemical enzymatic results will provide an important basis for understanding the varied neurotoxicity of organophosphate esters.

b.c. Summary of Results and Publications

PROGRESS REPORT 8/1/83 - 8/31/85 (K0098 AT U. of Illinois)

(* = References specifically acknowledging DOD support)

1.* K. Taira, T. Fanni, and D. G. Gorenstein, "Stereoelectronic Effects in the Hydrolysis of Methyl Ethylene Phosphate," J. Am. Chem. Soc. 106, 1521-1523 (1984).

2.* K. Taira, T. Fanni, and D. G. Gorenstein, "Stereoelectronic Effects in the Hydrolysis of Ethyl and Methyl Ethylene Phosphates," J. Org. Chem. 49, 4531-4536 (1984).

We have obtained evidence against the involvement of a hexacoordinate phosphorus intermediate in the alkaline hydrolysis of methyl and ethyl ethylene phosphate. An ^{18}O isotope effect on the ^{31}P chemical shift of the products of hydrolysis of the five-membered cyclic phosphate has been used to show that it

hydrolyzes with 100% P-O cleavage to give only the endocyclic product, alkyl-2-hydroxyethyl phosphate with only one ^{18}O atom incorporated from water. (Hence disproving the involvement of a hexacovalent intermediate). A much slower reaction involving recyclization of the methyl hydroxyethyl phosphate to form ethylene phosphate has been monitored by ^{31}P NMR. This slower reaction demonstrates that the endocyclic cleavage is favored by a significant factor over exocyclic cleavage. These results have given strong support to our hypothesis of stereoelectronic control in this reaction.

3.* J. C. Yang, A. Chang, and D. G. Gorenstein, ''A Reaffirmation of the Stereoelectronic Effect in the Hydrolysis of Ethyl and Methyl Ethylene Phosphates,'' to be submitted.

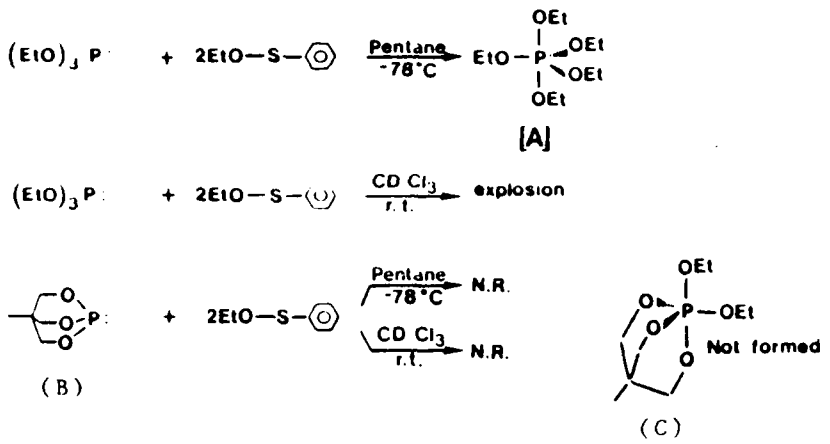
As described in Progress Report #1 and 2, we found that ethyl and methyl ethylene phosphates hydrolyze with complete endocyclic cleavage between pH 8 and 15 to yield ethyl and methyl 2-hydroxyethyl phosphates 3, respectively. Kluger and Thatcher (1985) have recently repeated these experiments and have suggested that MeOH is released and thus that cyclic cleavage does indeed occur in concentrated alkali. However, we have now established that most if not all of the MeOH released in their study is due to a dimerization reaction that occurs under their experimental conditions (methyl ethylene phosphate concentrations of .3 - .66 M). At our original and lower concentration ($\lesssim$ .02 M), this dimerization reaction is largely eliminated. The stereoelectronic effect is estimated to be at least 3 kcal/mol, in support of our earlier work.

4.* K. Taira and D. G. Gorenstein, ''Stereoelectronic Effects on the Basicity and Nucleophilicity of Phosphites and Phosphates. Ab Initio Molecular Orbital Calculations and the α-Effect,'' **J. Am. Chem. Soc. 106**, 7825-7831 (1984).

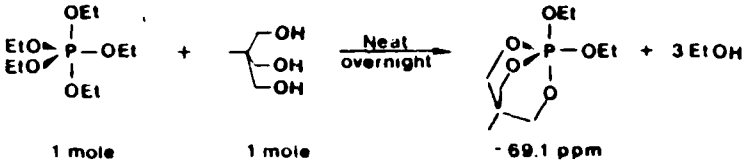
Ab Initio molecular orbital calculations have provided support for a stereoelectronic effect on the basicity and nucleophilicity of phosphites. In the phosphites, an antiperiplanar lone pair on oxygen to the phosphite lone pair raises the energy of the molecule by 3.5 kcal/mol relative to phosphite conformations with no app lone pairs to the phosphite lone pair. Upon phosphorus protonation of the phosphite the relative energy difference between the conformations reverses. The conformation with antiperiplanar lone pairs on oxygen to the P-H bond is now more stable than the conformation without this antiperiplanar lone pair interaction. Finally, the origin of the α effect, the enhanced nucleophilicity of a base possessing a heteroatom with an adjacent unshared electron pair is suggested to arise from a transtion state stereoelectronic effect. Whereas app oxygen lone pairs to the $P\text{-}H^+$ in the ground protonated phosphite stabilized the structure by only 1 kcal/mol at a P-H distance of 1.4 Å this stereoelectronic stabilization rises to > 12 kcal/mol, falls off again at even longer P-H bond distances, and finally reverses energies for the two conformations of the neutral phosphites.

5.* K. Taira, W. L. Mock, and D. G. Gorenstein, ''Experimental Tests of the Stereoelectronic Effect at Phosphorus: Nucleophilic Reactivity of Phosphite Esters,'' **J. Am. Chem. Soc. 106**, 7831-7835 (1984).

Triethyl phosphite rapidly reacts with ethyl benzenesulfenate or diethyl peroxide to yield pentaethoxyphosphorane, [A]. In contrast, 1-methyl-4-phospha-3,5,8-trioxabicyclo[2.2.2]octane [B], fails to react with either electrophile to yield the expected bicyclic phosphorane [C].

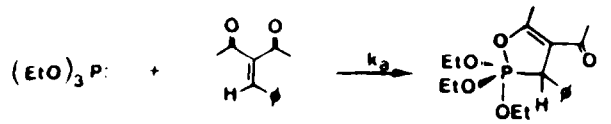


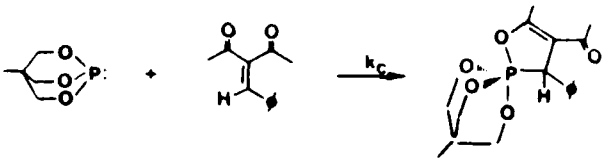
The poor reactivity of the bicyclic phosphite [B] is due to a kinetic rather than a thermodynamic barrier, because [C] is formed smoothly from an equimolar mixture of P(OEt)$_3$ and the triol, 1,1,1-tris(hydroxymethyl)ethane. This result is interpreted in terms of the stereoelectronic effect.



6.* K. Taira and D. G. Gorenstein, ''Experimental Tests of the Stereoelectronic Effect at Phosphorus,'' **Tetrahedron 40**, 3215 (1984).

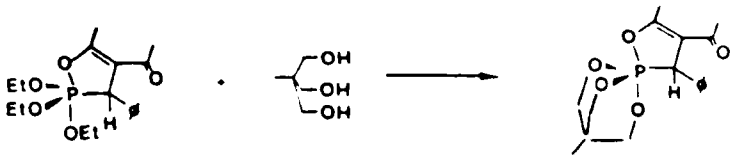
The order of nucleophilic reactivity of trialkyl phosphites with benzoyl chloride and 3-benzylidene 2,4-pentanedione is shown to be consistent with the stereoelectronic effect. Whereas triethyl phosphite readily reacts to yield the Michaelis-Arbusov product, diethyl benzoyl phosphonate, 1-methyl-4-phospha-3,4,8-trioxabicyclo[2.2.2]octane, [B], is essentially unreactive, even at a higher temperature. The bicyclic phosphite, [B], also reacted 750 times slower than the equatorial 2-methoxy ester of a 1,3,2-dioxaphosphorinane, in a Michael addition reaction with 3-benzylidene-2,4-pentanedione. (Triethyl phosphite reacts with intermediate reactivity).



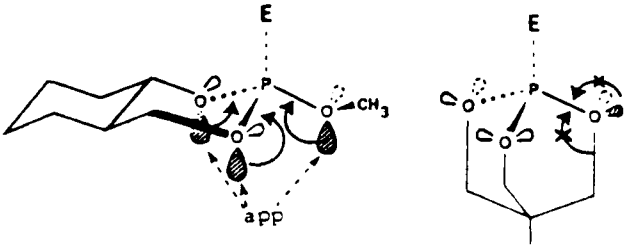


$k_a >> k_c$

This is also a kinetic effect since the bicyclic phosphite ketone adduct is thermodynamically more stable:



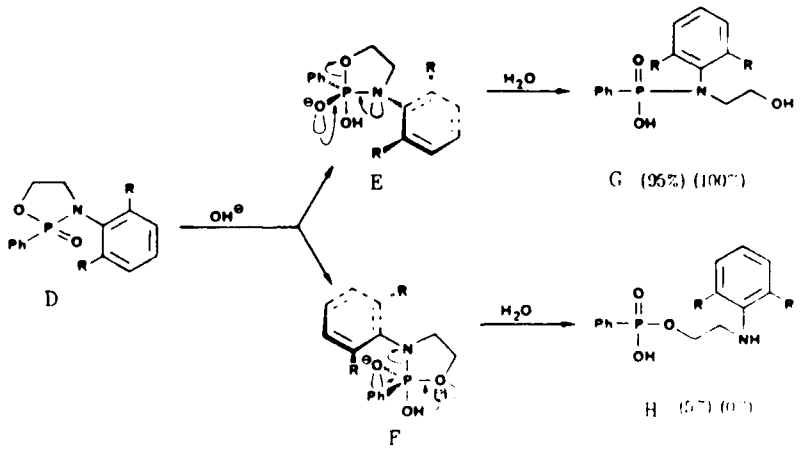
As expected from the stereoelectronic effect, the nucleophile with the largest number of app lone pairs on oxygen to the incipient P-C bond has the highest reactivity, and the bicyclic phosphite without any app lone pairs reacts slowest.



7. J. C. Yang and D. G. Gorenstein, "Stereoelectronic Control in the Base Catalyzed Hydrolysis of Five-Membered Ring Cyclic Phosphoramidates," **Tetrahedron Lett. 25, 4627-4630 (1984).**

Stereoelectronic effects were shown to strongly affect the P-O vs P-N cleavage pathway of five-membered ring phosphoramidates. As shown in Scheme I, cyclic phosphoramidates [D](R=Me) and [D](R=iPr), hydrolyze in base to give 95% and 100% P-O cleavage products, respectively:

Scheme I



Alkyl substitution dramatically reverses the 100% P-N cleavage observed for the unsubstituted phosphoramidate [D] (R=H). When the 2,6-positions of the aromatic ring are substituted by alkyl groups ([D] R=Me and i-Pr), the

hybridization of the nitrogen can no longer be sp^2 since the conjugation with the aromatic ring will be destroyed by severe steric interactions between the alkyl substituents on the aromatic ring and the cyclic five-membered ring. Phosphoranes [E] (R=Me, iPr) formed by hydroxide attack opposite the ring oxygen will now have two lone pairs app to the scissile apical oxygen bond, one from oxygen and the other at least partially from the sp^3-hybridized nitrogen. P-O bond cleavage will thus be stereoelectronically feasible in [D] (R=Me, iPr). In the unsubstituted [D] (R=H), the nitrogen lone will not be app to the scissile P-O bond in [E] since it will be in the basal plane of the trigonal bipyramid phosphorane (sp^2-hybridized N atom).

8.* D. G. Gorenstein and K. Taira, ''Stereoelectronic Control in Peptide Bond Formation. Ab Initio Calculations and Speculations on the Mechanism of Action of Serine Proteases,'' **Biophys. J. 46**, 749-762 (1984).

We have completed an ab initio molecular orbital calculation on the stereoelectronically controlled reaction surface for the hydrolysis of formamide. Calculated transition state energies for the first addition step of the reaction reveals that a lone pair on the oxygen of the OH group which is antiperiplanar to the attacking nitrogen stabilizes the transition state by 3.9 kcal/mol, thus supporting the hypothesis of stereoelectronic control for this reaction.

9. R. O. Day, D. G. Gorenstein, and R. R. Holmes, ''Crystal Structure of an Axially and Equatorially Oriented 2-aryloxy-2-oxy-1,3,2-dioxaphosphorinane,'' **Inorg. Chem. 22**, 2192 (1983).

X-ray studies (in collaboration with R. Holmes, U. Mass.) on our epimeric six-membered ring phosphate triesters 1 have confirmed the proposed structures and some of our predictions of bond angle and torsional angle effects on ^{31}P chemical shifts.

10. D. Shah, D. Kallick, R. Rowell, R. Chen, and D. G. Gorenstein, ''Stereochemistry of the Phosphorylation Reaction of α-Chymotrypsin by a Cyclic Phosphate Triester,'' **J. Am. Chem. Soc. 105**, 6942 (1983).

11. D. Kallick, D. O. Shah, and D. G. Gorenstein, ''^{31}P NMR of Covalent Phosphorylated Derivatives of α-Chymotrypsin,'' **Bull. Magn. Reson. 5**, 251 (1983).

12. D. G. Gorenstein, ''Non-Biological Aspects of P-31 NMR Spectroscopy,'' **Progress in NMR Spectroscopy 161**, 1-98 (1983).

13. D. G. Gorenstein (editor), **P-31 NMR: Principles and Applications**, Academic Press, 1984).

14. D. G. Gorenstein and D. Shah, ''Selective Compilation of P-31 NMR Data,'' In **P-31 NMR: Principles and Applications**, Chapter 19, Academic Press, 1984).

15. D. G. Gorenstein, ''P-31 Chemical Shifts,'' In **P-31 NMR: Principles and Applications**, Chapter 2, Academic Press, 1984.

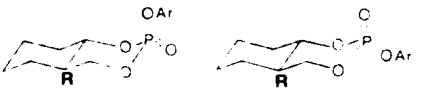
16. D. G. Gorenstein, ''P-31 Coupling Constants,'' In **P-31 NMR: Principles and Applications**, Chapter 3, Academic Press, 1984.

17. D. G. Gorenstein, ''Introduction to ^{31}P NMR in Solution: Theory and Experiment,'' introductory chapter In **Topics in Phosphorus Chemistry** (M. Grayson, Ed.) in press, 1986.

Our ^{31}P NMR studies on organophosphorus compounds and enzyme complexes supported by DOD have allowed us to develop a stereoelectronic effect on ^{31}P chemical shifts and $^{1}J_{PX}$ coupling constants. This work was also partially supported by NIH (GM-17575).

18.* K. Taira, K. Lai, and D. G. Gorenstein, ''Stereoelectronic Effects in the Conformation and Hydrolysis of Epimeric (4aα, 8aβ-Hexahydrobenzo-2-(p-Nitrophenoxy)-2-Oxo-1,3,2λ^5-Dioxaphosphorinanes and 4aα-methyl-8aβ-Pentahydrobenzo-2-(p-Nitrophenoxy)-2-Oxo-1,3,2λ^5-Dioxaphosphorinanes,'' **Tetrahedron**, in press.

The hydrolysis of (4aα, 8aβ)-hexahydrobenzo-2-(p-nitrophenoxy)-2-oxo-1,3,2λ^5-dioxaphosphorinanes (axial and pseudo-equatorial p-nitrophenoxy group) and 4aα-methyl-8aα-pentahydrobenzo-2-(p-nitrophenoxy)-2-oxo-1,3,2λ^5-dioxaphosphorinanes

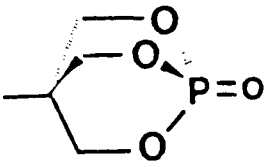


1 (R = CH_3)

(axial and equatorial p-nitorphenoxy group) 1 were shown to be consistent with the idea that the axial isomers react via a chair conformation and the equatorial isomer 1b reacts via a twist-boat conformation with the leaving group in a pseudo-axial position. This supports earlier stereoelectronic effects observed in this ring system.

19.* T. Fanni, K. Taira, D. G. Gorenstein, R. Vaidyanathaswamy, and J. G. Verkade, ''Stereoelectronic Effects in the Hydrolysis of Bicyclic and Acyclic Phosphates and Phosphorothionates,'' submitted.

The bicyclic phosphate 1-methyl-4-phospha-3,5,8-trioxabicyclo[2.2.2]octane-4-oxide, [I], hydrolyzes at pH = 14, 5.2 x 10^3 times faster than the acyclic compound triethyl phosphate. Similarly the bicyclic phosphorothionate 1-methyl-4-phospha-3,5,8-trioxabicyclo[2.2.2]octane-4-sulfide, [J], hydrolyzes 8.1 x 10^2 times faster



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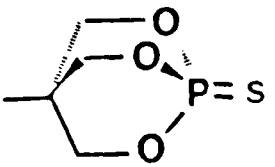
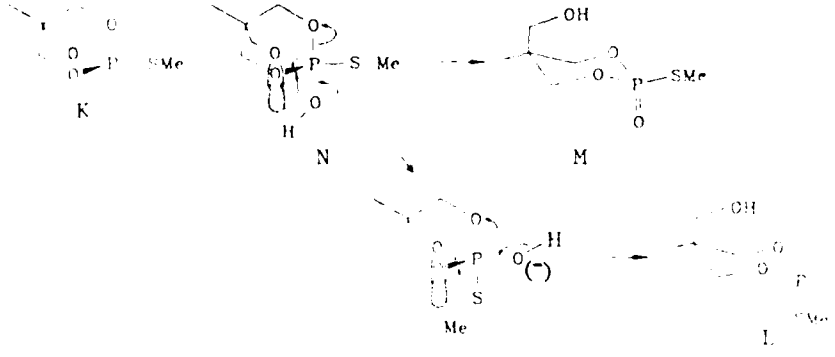


J

than the acyclic compound triethyl phosphorothionate. Part of this rate enhancement is attributed to a stereoelectronic factor since compounds [I] and [J] have two antiperiplanar (app) lone pairs to the breaking P-O bond, while in order to put two app lone pairs to the breaking P-O bond in the acyclic compounds, the molecule has to be constrained to a proper conformation which is disfavored entropically.

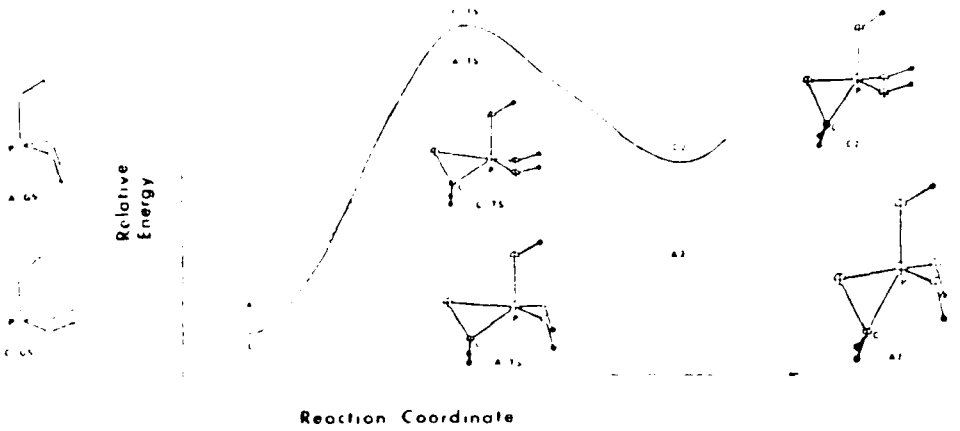
20.* K. Taira, T. Fanni, and D. G. Gorenstein, ''Stereoelectronic Effects in the Formation and Hydrolysis of Phosphonium Ions from Acyclic and Bicyclic Phosphorothionates,'' to be submitted.

The alkaline hydrolysis of the bicyclic phosphonium ion 1-methyl-3,5,8-trioxabicyclo-[2.2.2]octane-4-methylthiophosphonium triflate [K] proceeds mainly with P-O bond cleavage as shown by hydrolysis in ^{18}O labeled water to form the cyclic epimers [L] and [M] in addition to the bicyclic phosphate [I]. Epimer [L] is formed after pseudorotation of the trigonal bipyramid (tbp) intermediate [N] and is the predominant product. This could be explained by fast pseudorotation to give the more stable tbp in which the negatively charged oxygen is placed in a basal position. In contrast the acyclic analog triethoxy methyl thiophosphonium ion undergoes alkaline hydrolysis with 100% P-S bond cleavage. These results are in accord with the stereoelectronic effect.



21.* A. Chang, U. Shiga, K. Taira, and D. G. Gorenstein, ''Stereoelectronic Effects on the Addition of Phosphites to the Carbonyl Double Bond. Ab Initio Molecular Orbital Calculations and the α-Effect,'' submitted.

A theoretical analysis of the carbonyl addition reaction of acyclic and bicyclic phosphites has been carried out. Formaldehyde was chosen as the electrophile and trihydroxy phosphine ($:P(OH)_3$) as the nucleophile. Reaction surfaces were then calculated for different orientations about the P-OH bond in trihydroxy phosphine representing the geometries of bicyclic (I) and acyclic phosphites. Most importantly, during the cleavage of the P-C bond in the stable pentacovalent phosphorane adduct $(HO)_3P{\cdot}H_2C{=}O$, transition states leading to 1,3-dipolar intermediates are clearly established for both geometries. These results support our emphasis that the kinetic stereoelectronic effect and the α-effect are related and largely transition state phenomena.



22. D. G. Gorenstein, ''P-31 NMR of tRNA'' In **P-31 NMR: Principles and Applications**, Chapter 10, Academic Press, 1984.

23. D. G. Gorenstein, ''P-31 NMR of Drug-Nucleic Acid Complexes,'' In **P-31 NMR: Principles and Applications**, Chapter 11, Academic Press, 1984.

24. D. G. Gorenstein and E. Goldfield, ''P-31 NMR of Nucleic Acids,'' **Bull. Magn. Reson. 5**, 161 (1983).

Peripherally related to our DOD ^{31}P NMR studies (largely funded by NIH) but developed through our DOD calculations and ^{31}P work, we have applied our proposed stereoelectronic theory of ^{31}P chemical shifts to biological systems.

Thus additional support for the stereoelectronic ^{31}P shift hypothesis was provided by ^{31}P NMR studies on nucleic acids and discussed in several of the above review articles I wrote on the structural basis of ^{31}P chemical shifts.

During this period 14 other articles (see C.V. at end) were also published on these biological aspects of ^{31}P NMR. (supported entirely by NIH).

25.* K. Taira, A. Chang, J. C. Yang, and D. G. Gorenstein, ''Stereoelectronic Effects in Organophosphorus Chemistry,'' **Tetrahedron**, to be submitted.

We have been invited to write a review article covering the entire field of stereoelectronic effects in organophosphorus chemistry.

26.* D. G. Gorenstein, K. Taira, B. A. Luxon, J. B. Findlay, D. Kallick, and D. Shah, ''Stereoelectronic Control in the Enzymatic and Non-Enzymatic Reactions of Organophosphorus Compounds,'' CRDC Scientific Conference on Chemical Defense Research, 1983.

Both theory and experiment provide support for the stereoelectronic effect in the organic and enzymatic reactions of organophosphorus compounds. Ab initio molecular orbital calculations on the reaction of dimethyl phosphate with hydroxide to yield monomethyl phosphate and methoxide show than an antiperiplanar lone pair to the scissile bond lowers the transition state energy by 7-11 kcal/mol. These

theoretical predictions are confirmed in the reactions of conformationally restricted phosphorus compounds. Stereoelectronic effects appear to play a role in the phosphorylation of the serine esterase, α-chymotrypsin.

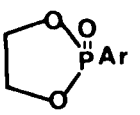
27.* D. G. Gorenstein, K. Taira, A. Chang, and J.-C. Yang, ''Stereoelectronic Control in Enzymatic and Non-Enzymatic Acyl and Phosphoryl Transfer Reactions,'' CRDC Scientific Conference on Chemical Defense Research, 1985.

Ab initio molecular orbital calculations have been performed on the reaction profile for the addition/elimination reaction between ammonia and formic acid, proceeding via a tetrahedral intermediate: $NH_3 + HCO_2H \rightarrow H_2NCH(OH)_2 \rightarrow NH_2CHO + H_2O$. Calculated transition state energies for the first addition step of the reaction revealed that a lone pair on the oxygen on the OH group, which is antiperiplanar to the attacking nitrogen, stabilized the transition state by 3.9 kcal/mol, thus supporting the hypothesis of stereoelectronic control for this reaction and suggesting the importance of stereoelectronic effects for the mechanism of action of serine proteinases.

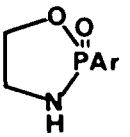
Ab initio molecular orbital calculations as well as experiment on various model systems have also been carried out on cyclic phosphite and phosphate esters. Again significant stereoelectronic effects have been revealed. New data on the alkaline hydrolysis of methyl ethylene phosphate confirm our original prediction of important stereoelectronic effects.

28.* C. J. Yang and D. G. Gorenstein, ''Experimental Separation of Ring Strain and Stereoelectronic Effects on Cyclic Five-Membered Ring Phosphorus Esters,'' in preparation.

2-Phenyl-2-oxo-aza and dioxaphospholanes [O] and [P] and their acyclic analogs have been prepared and the rates and activation parameters of their alkaline



[O]

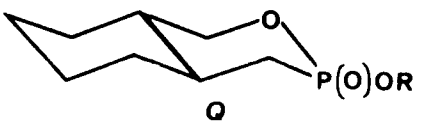


[P]

hydrolysis measured.

29.* A. Chang and D. G. Gorenstein, ''Stereoelectronic Effects in the Hydrolysis of Phosphonate Esters,'' in preparation.

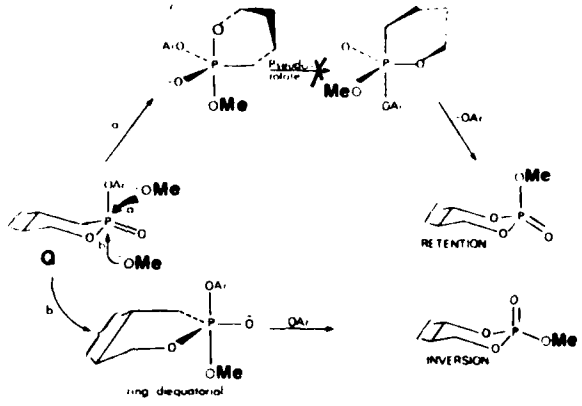
An attempt to restrict the chair to twist-boat phosphorinane ring flipping was made by replacing one of the ring oxygen atoms in [1] by a methylene group [Q].



Q

This replacement causes 1,3-steric and eclipsing interactions from the two hydrogen atoms of the methylene group in the equatorial isomer. These interactions disfavor the twist-boat conformation and hence prevent the chair conformation from flipping in the ground state.

Methanolysis of the epimeric phosphonate diesters in dimethyl formamide showed exclusively inversion products for both axial and equatorial isomers. In contrast to the phosphate triesters, 1 (R=H, CH_3)the absence of any retention products is attributed to the high barrier for pseudorotation of phosphonate [Q] in the trigonal bipyramid intermediates.

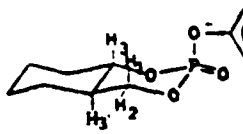
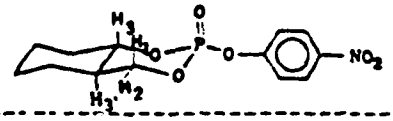
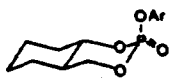
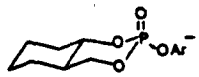
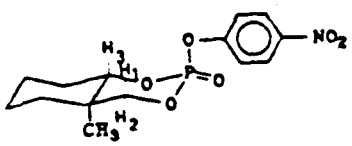
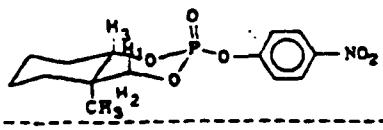
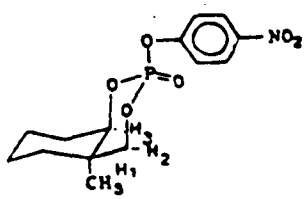


Alkaline hydrolysis of the epimeric phosphonate diesters established that once again the rate difference for hydrolysis of the two epimers is attributed to ground state energy differences and that hydrolysis proceeds via similar transition states for both equatorial and axial isomers. The transition states for both isomers are suggested to have the six membered ring in a half-chair di-equatorial conformation. The basal ring oxygen lone pair in the transition state can overlap with the entering and leaving groups, again maximizing the stereoelectronic effect.

30.* D. Kallick, K. Taira, M. Miyazaki, and D. Gorenstein, ''Organophosphate Inhibition of α-Chymotrypsin,'' in preparation.

The relative rates of enzymatic and non-enzymatic reaction of some bicyclic dioxaphosphorinanes have also been determined. Preliminary results are shown in Table I. Clearly α-chymotrypsin has a stereochemical preference for certain stereoisomers. Rate constants for irreversible inhibition of the α-chymotrypsin and enzyme catalyzed hydrolysis of these esters are now being completed (D. Kallick, Progress Report 35).

Table I. Relative rates of α-chymotrypsin irreversible inhibition to non-enzymatic hydrolysis of phosphorinane triesters.

Substrate	k_E/k_H*
	0.44
	1.08
	69.0
	9.43
	1.0
	5.86
	0.62

*Initial enzymatic rates (k_E) and non-enzymatic hydrolysis in 12% MeOH, 30°C, pH 7.86, tris buffer. $[E]_o$ = 3.91 mM, $[S]_o$ = 3.91 mM.

PNP = p-nitrophenyl; Ar = 2,4-dinitrophenyl.

31.* K. Taira, ''Theoretical and Experimental Evidence for the Stereoelectronic Effect,'' Ph.D. Thesis, Univ. of Illinois at Chicago, 1984.

32.* T. Fanni, ''Stereoelectronic Effects in Organophosphorus Chemistry,'' Ph.D. Thesis, Univ. of Illinois at Chicago, 1985.

33.* C. J. Chang, ''Stereoelectronic Effects in Five-Membered Ring Organophosphorus Compounds,'' Ph.D. Thesis, Univ. of Illinois at Chicago, 1986.

34.* A. Chang, ''Theoretical and Experimental Studies on the Stereoelectronic Effect,'' Ph.D. Thesis, Univ. of Illinois at Chicago, 1986.

35.* D. Kallick, ^{31}P and Multinuclear NMR Studies of Transition-State Analog Complexes of Serine Proteinases and Esterases,'' Ph.D. Thesis, Univ. of Illinois at Chicago, 1986.

c. Technical Reports

Progress Report #1 8/1/83-12/31/83
Progress Report #2 1/1/84-6/30/84
Progress Report #3 7/1/84-12/31/84
Progress Report #4 1/1/85-6/30/85
Progress Report #5 7/1/85-12/31/85

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*No ARO direct support of salary

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